

DETERMINING A GENETIC DISTANCE

The classical method for determining the genetic distance between the loci of two allele pairs known to reside on the same homologous chromosome pair of an organism involves observing the phenotypes of the offspring of one of two particular breedings. During the course of Thomas Hunt Morgan's work on fruit flies, he happened to carry out both breedings and was rewarded not only with the first clear evidence of crossing over but also with the first unambiguous genetic-distance data. Morgan's experiments and data are used here to illustrate the procedure.

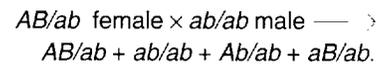
The allele pairs in question reside on one of the homologous autosome pairs of *Drosophila melanogaster*. One allele pair affects eye color: a dominant allele *A* that specifies red eye color and a recessive allele *a* that specifies purple eye color. The other allele pair affects wing length: a dominant allele *B* that specifies wild-type wings and a recessive allele *b* that specifies vestigial (very short) wings.

The participants in the first breeding are a female fruit fly that is heterozygous for both traits (and therefore has red eyes and normal wings) and a male fruit fly that is homozygous for both recessive trait variants (and therefore has purple eyes and vestigial wings). Furthermore, the female is known to be a product of the breeding $AABB \times aabb$. Therefore the distribution of the alleles *A*, *a*, *B*, and *b* on the homologous autosome pair of the female is known: Both dominant alleles (*A* and *B*) reside on one member of the homologous autosome pair, and both recessive alleles (*a* and *b*) reside on the other member. Such an allele distribution is denoted by writing the genotype of the female as AB/ab . The distribution of the alleles *a*, *a*, *b*, and *b* on the homologous autosome pair of the male is also known (because the male is homozygous for both traits) and is denoted in a similar fashion as ab/ab . Thus the first breeding can be symbolized by



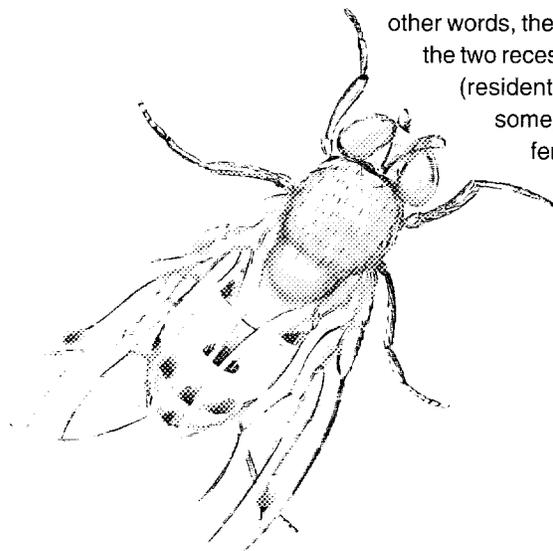
Meioses in the heterozygous female that involve no crossovers between the two loci yield two types of eggs: those possessing the chromosome with the allele combination AB and those possessing the chromosome with the allele combination ab . In other words, the two dominant alleles and the two recessive alleles remain linked (resident on the same chromosome), just as they are in the female herself. But those

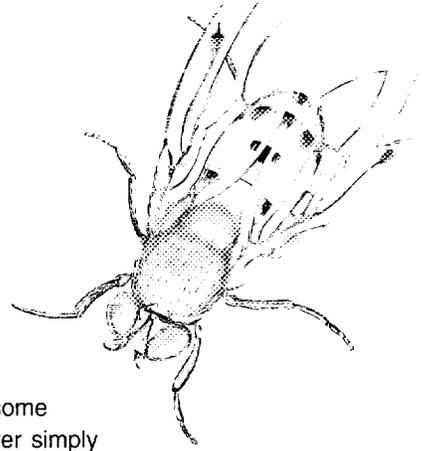
meioses in the female that involve a single crossover between the two loci (or any odd number of crossovers) yield in addition two other types of eggs: those possessing a chromosome with the allele combination Ab and those possessing a chromosome with the allele combination aB . In other words, a single crossover between the two loci establishes linkage between one dominant and one recessive allele. On the other hand, meioses in the doubly homozygous male, whether or not they involve crossovers between the two loci, yield sperms possessing only the allele combination ab . Thus the offspring of breeding 1 possess four genotypes, each corresponding to one of the four possible phenotypes:



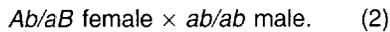
Morgan examined more than 2800 progeny of breeding 1 and found that 47.2 percent had red eyes and normal wings (AB/ab), 42.1 percent had purple eyes and vestigial wings (ab/ab), 5.3 percent had red eyes and vestigial wings (Ab/ab), and 5.4 percent had purple eyes and normal wings (aB/ab). All the offspring exhibiting the last two phenotypes (the combinations of one recessive trait variant and one dominant trait variant) result only from crossovers during meioses in the female parent. Thus the data indicate that the probability of new allele linkages being formed by crossing over is $0.107 = 0.053 + 0.054$. That value for the so-called recombination fraction corresponds to a genetic distance of about 12 centimorgans. (The relationship between recombination fraction and genetic distance is presented in "Classical Linkage Mapping" in "Mapping the Genome.")

The participants in the other breeding that provides unambiguous recombination-fraction data are, like the participants in breeding 1, a doubly heterozygous female and a doubly homozygous-recessive male. How-

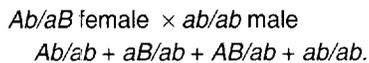




ever, the second female is known to be a product of the breeding $Ab/Ab \times aB/aB$ (rather than the breeding $AB/AB \times ab/ab$). Therefore the distribution of alleles on her homologous autosome pair is Ab/aB (rather than AB/ab). (The difference in allele distributions of the two doubly heterozygous females is often referred to as a difference in linkage phase.) The second breeding is thus symbolized by



Breeding 2 yields offspring that exhibit the same genotypes and phenotypes as breeding 1:



Morgan examined more than 2300 progeny of breeding 2 and found that 41.3 percent had red eyes and vestigial wings (Ab/ab), 45.7 percent had purple eyes and normal wings (aB/ab), 6.7 percent had red eyes and normal wings (AB/ab), and 6.3 percent had purple eyes and vestigial wings (ab/ab). Again, all the offspring exhibiting the last two phenotypes result only from crossovers during meioses in the female parent. Thus the data indicate that the recombination fraction for the two allele pairs is 0.130, which corresponds to a genetic distance of about 15 centimorgans.

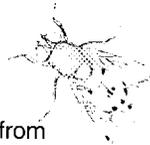
Note that the two data sets yield different values for the same genetic distance. However, the difference between the values is within the statistical uncertainties associated with measurements of probabilistic events. Note also that the same genetic distance could in principle be determined by carrying out the reciprocal of breeding 1 or breeding 2 (that is, a breeding between a doubly heterozygous male and a doubly homozygous-recessive female). Then, the crossovers detected are those that occur

during meioses in the male parent rather than in the female parent. However, for some unknown reason crossing over simply does not occur in male fruit flies. But fruit flies are exceptional in that respect, and genetic distances for other species can be determined by carrying out either breeding 1, say, or its reciprocal.

Breedings 1 and 2 are those that provide unambiguous recombination-fraction data. As an example of the ambiguities that can arise, consider the fruit-fly breeding



Assume first that crossing over between the two loci does not occur during meioses in the female parent. Then the offspring of breeding 3 exhibit two phenotypes: red eyes and normal wings (AB/AB and AB/ab) and purple eyes and vestigial wings (ab/ab). Now assume that crossing over does occur during meioses in the female parent. Then among the offspring of breeding 3 are some that exhibit the two other possible phenotypes: red eyes and vestigial wings (Ab/ab) and purple eyes and normal wings (aB/ab). All offspring that exhibit those two phenotypes result only from crossing over. However, crossing over also leads to offspring that exhibit one of the phenotypes produced in the absence of crossing over, namely, red eyes and normal wings (Ab/AB and aB/AB). In other words, whereas the offspring produced by breeding 1 or 2 can unambiguously be sorted by phenotype into two categories—those that are the result of crossovers and those that are not—the offspring resulting from breeding 3 cannot be so sorted because meioses that do and do not involve crossovers result in the doubly dominant phenotype.



The reader can accept on faith or verify personally that breedings 1 and 2 are the only breedings that provide unambiguous recombination-fraction and hence genetic-distance data. Note, in addition, that obtaining even ambiguous data requires that one parent be doubly heterozygous.

Determining a genetic distance is thus relatively easy when the breeding of the organism in question can be manipulated at will. But determining the genetic distance between the loci of two human allele pairs is much more difficult, since the breeding of humans cannot be manipulated, the genotypes and allele distributions of human parents are not always known, and human breedings generally produce so few offspring that the statistical uncertainty in the measured recombination fraction is large.